US009441275B2

(12) **United States Patent**
Sadee et al.

(10) **Patent No.: US 9,441,275 B2**
(45) **Date of Patent: Sep. 13, 2016**

(54) **POLYMORPHISM IN CYP3A4 GENE AFFECTING DRUG METABOLIZING AND USES THEREOF**

(75) Inventors: **Wolfgang Sadee**, Upper Arlington, OH (US); **Danxin Wang**, Columbus, OH (US)

(73) Assignee: **Ohio State Innovation Foundation**, Columbus, OH (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 61 days.

(21) Appl. No.: **13/259,514**

(22) PCT Filed: **Mar. 26, 2010**

(86) PCT No.: **PCT/US2010/028842**

§ 371 (c)(1), (2), (4) Date: **Oct. 7, 2011**

(87) PCT Pub. No.: **WO2010/111600**

PCT Pub. Date: **Sep. 30, 2010**

(65) **Prior Publication Data**

US 2012/0040347 A1 Feb. 16, 2012

Related U.S. Application Data

(60) Provisional application No. 61/163,555, filed on Mar. 26, 2009.

(51) **Int. Cl.**

C12P 19/34	(2006.01)
C12Q 1/68	(2006.01)
A01N 61/00	(2006.01)
G01N 33/50	(2006.01)

(52) **U.S. Cl.**

CPC ***C12Q 1/6883*** (2013.01); ***G01N 33/5038*** (2013.01); *C12Q 2600/106* (2013.01); *C12Q 2600/16* (2013.01); *C12Q 2600/172* (2013.01); *G01N 2800/044* (2013.01); *G01N 2800/52* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2003/0215819	A1	11/2003	Frudakis
2006/0073479	A1	4/2006	Frudakis
2008/0292584	A1	11/2008	Roberts et al.

FOREIGN PATENT DOCUMENTS

WO	0120025	A2	3/2001
WO	2010111600	A1	9/2010

OTHER PUBLICATIONS

Hegele, Arterioscler. Thromb. Vasc. Biol. 2002, 22:1058-1061.*

PL Detail-Document 250801, Statin Dose Comparisons, Pharmacist's Letter/Prescribers Letter, May 2012, pp. 1-12.*

Gao (Eur J Clin Pharmacol, 2008, 64:877-882).*

PCT International Preliminary Report on Patentability, PCT/US2010/028842 filed Mar. 26, 2010, dated Oct. 6, 2011.

PCT International Search Report and the Written Opinion, PCT/US2010/28842 filed Mar. 26, 2010, dated Aug. 12, 2010.

GenBank, Reference SNP (refSNP) Cluster Report: rs 35599367, dated Dec. 20, 2008, Retrieved from the Internet:<URL:http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=35599367>, pp. 1-2.

Yamada, Y. et. al., "Genetic Risk for Metabolic Syndrome: Examination of Candidate Gene Polymorphisms Related to Lipid Metabolism in Japanese People," J. Med. Genet., Jan. 2008, pp. 22-28, vol. 45, No. 1.

* cited by examiner

Primary Examiner — Sarae Bausch

(74) *Attorney, Agent, or Firm* — Meunier Carlin & Curfman LLC

(57) **ABSTRACT**

A method for predicting a subject's risk factors for CYP3A4-related disorders includes detecting the allelic status of a SNP in a nucleic acid sample of the subject.

12 Claims, 3 Drawing Sheets
(1 of 3 Drawing Sheet(s) Filed in Color)

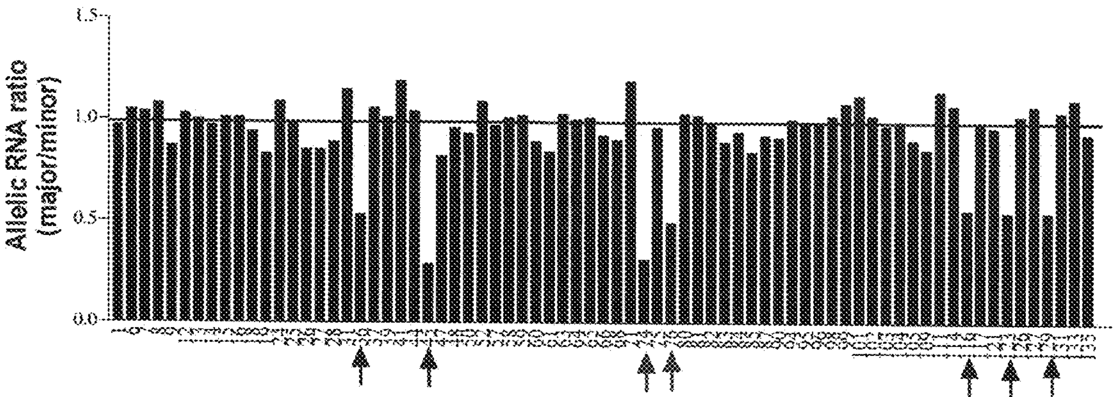

Figure 1A

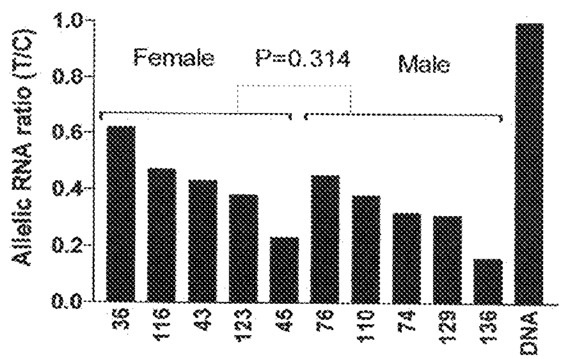

Figure 1B

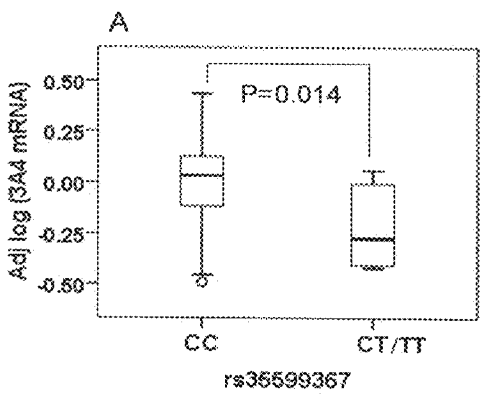

Figure 2A

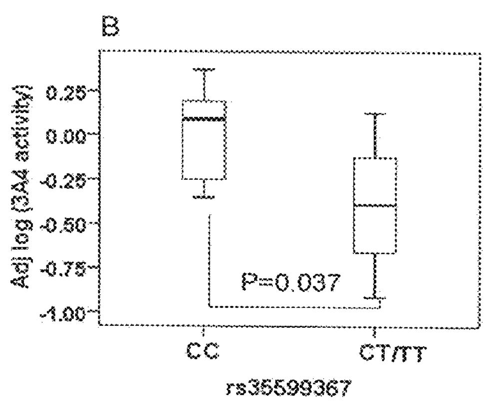

Figure 2B

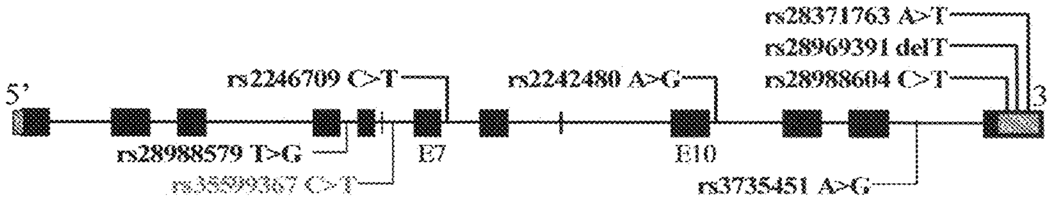

Figure 3

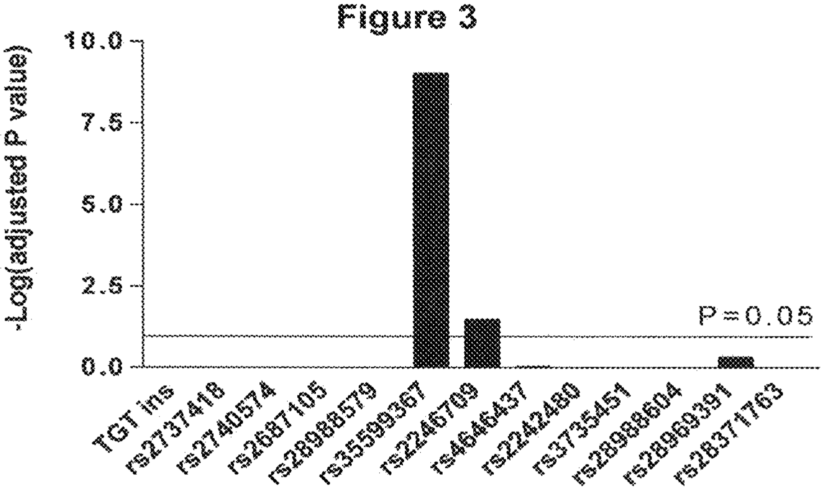

Figure 4

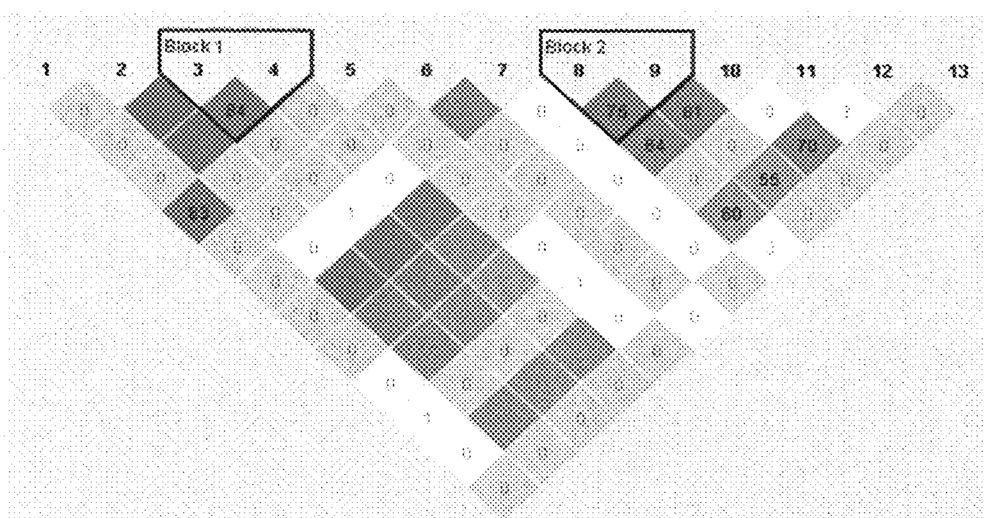

Figure 5

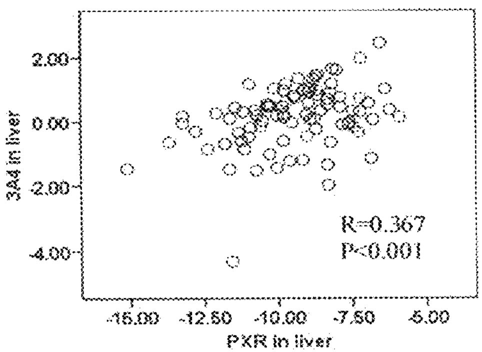

Figure 6A

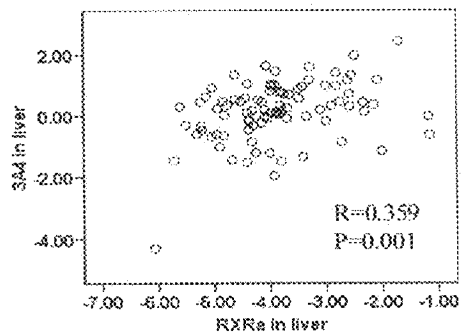

Figure 6B

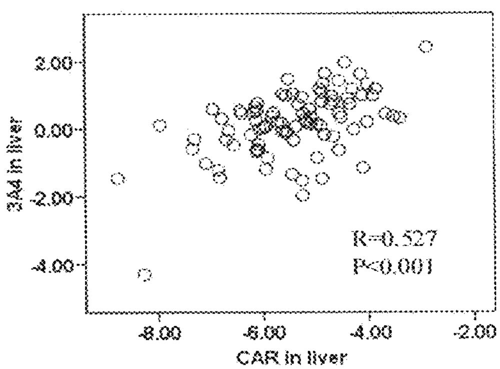

Figure 6C

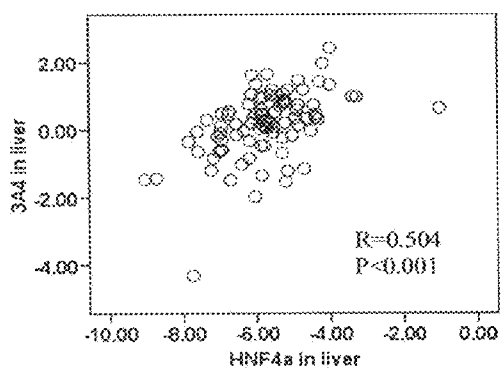

Figure 6D

POLYMORPHISM IN CYP3A4 GENE AFFECTING DRUG METABOLIZING AND USES THEREOF

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application 61/163,555 filed Mar. 26, 2009, the disclosure of which is incorporated herein by reference, in its entirety.

GOVERNMENT SUPPORT

This invention was made with government support under Grant Number NIH NIAID (1R21AI074399) awarded by the National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. The ASCII copy, created on Mar. 23, 2010, is named 604_50806_SEQLIST_OSURF-09032.txt and is 34,669 bytes in size.

BACKGROUND

Cytochrome P450 (CYP) enzymes metabolize endogenous and xenobiotic compounds. CYP3A4 belongs to the CYP3A subfamily and is the most abundant CYP enzyme. CYP3A4 is involved in metabolizing 45-60% of all currently used drugs (1), including several statins—cholesterol-lowering HMG-CoA reductase inhibitors. However, CYP3A4 activity shows wide inter-individual variation, influencing drug response and toxicity. While genetic factors are thought to be main contributors to inter-individual differences in CYP3A4 activity (2), currently known CYP3A4 polymorphisms cannot account for the observed variability.

Genetic variants in CYP3A4 that change the amino acid sequence are rare (<1%). A more common variant, CYP3A4*1B, in the 5'-flanking region, has been associated with drug response and diseases (3,4), but results are inconsistent (5-7), and its function remains controversial (3,8-10). Moreover, CYP3A4*1B is in linkage disequilibrium (LD) with the adjacent CYP3A5 (11), encoding a similar but usually less abundant CYP enzyme that could have accounted for any linked clinical phenotype (12).

Further suspected CYP3A4 polymorphisms include a TGT insertion (13), an enhancer region SNP (rs2737418) (14), and an intron7 SNP (rs4646437) (15). While reporter gene assays suggested an effect for the TGT insertion and for rs2737418, the in vivo significance of TGT remains unresolved (13), while results on CYP3A4 mRNA and enzyme activity were contradictory for rs2737418 (14). The intron7 SNP rs4646437 was found to be associated with CYP3A4 protein/enzyme activity, but only in livers from males (15). Therefore, the role of functional polymorphisms in CYP3A4 remains uncertain.

Single nucleotide polymorphisms (SNPs) are useful as biomarkers for predicting disease susceptibility or progression, or as a guide for individualized therapy, including drug therapy.

What are lacking are tools for predicting the likelihood that a particular patient will be responsive to a particular therapeutic agent, and in particular, identifying polymorphisms to which a CYP3A4 agent will be sensitive or resistant. Also lacking are tools for profiling genetic factors influencing sensitivity and resistance of patients to such therapeutic agents. Such tools, and the resulting gene expression profiles, would be predictive of treatment response of a patient to a particular drug, and would allow for increased predictability regarding efficacy, adverse drug reactions, chemosensitivity or chemoresistance of such patients to enable the design of optimal treatment regimens for patients, or to enable drug development in early clinical trials avoiding unexpected toxicities in poor metabolizers.

Additional advantages, objects, and features of the invention will be set forth in part in the description which follows and in part will become apparent to those having ordinary skill in the art upon examination of the following or may be learned from practice of the invention. The objects and advantages of the invention may be realized and attained as particularly pointed out in the appended claims.

SUMMARY

In a first aspect, there is provided herein a method for predicting a subject's response to CYP3A4-metabolized compounds, including, for example efficacy and/or adverse drug reactions. The method includes detecting the allelic status of one or more polymorphisms in a nucleic acid sample of the subject, wherein the polymorphism is one or more of: i) CYP3A4-associated SNP (rs35599367 [SEQ ID NO: 152]) located in intron6 (C>T); or, ii) a SNP in linkage disequilibrium therewith, wherein the allelic status of the polymorphism in the subject is predictive of the subject's risk for having or developing the CYP3A4-related disorder.

In another aspect, there is provided herein, a method of screening a subject for a prognostic biomarker of an CYP3A4-related disorder, comprising detecting the allelic status of one or more polymorphisms in a nucleic acid sample of the subject, wherein the polymorphism is one or more of: i) CYP3A4-associated SNP (rs35599367 [SEQ ID NO: 152]) located in intron6 (C>T); or, ii) a SNP in linkage disequilibrium therewith, wherein the allelic status of the polymorphism in the subject is predictive of the prognostic outcome of the CYP3A4-related disorder.

In certain embodiments, the method further includes the step of correlating the allelic status of the polymorphism in the subject with the allelic status of the polymorphism in a reference population to predict the subject's risk for having or developing the CYP3A4-related disorder.

In certain embodiment, the method further includes the step of correlating the allelic status of the polymorphism in the subject with the allelic status of the polymorphism in a reference population to predict whether the subject has a more or less severe phenotype of the CYP3A4-related disorder.

In certain embodiment, the method further includes the step of correlating the allelic status of the polymorphism in the subject with the allelic status of the polymorphism in a reference population to predict the prognostic outcome of the disorder in the subject.

In certain embodiment, the method further includes the step of correlating the allelic status of the polymorphism in the subject with the allelic status of the polymorphism in a reference population to predict the subject's response to treatment, dosage and/or toxicity.

In certain embodiment, the CYP3A4-related disorder comprises a metabolic-related disorder.

In certain embodiment, the CYP3A4-metabolized compounds comprise one or more pharmaceuticals metabolized in the liver, including statin drugs.

In certain embodiment, the CYP3A4-metabolized compounds comprise one or more of: CYP3A4 inhibitors or CYP3A4 enhancers.

In certain embodiment, the polymorphism comprises a CYP3A4-associated SNP (rs35599367 [SEQ ID NO: 152]) located in intron6 (C>T).

In certain embodiment, the polymorphism comprises rs35599367 [SEQ ID NO: 152], wherein the presence of the polymorphism in a subject is predictive of an increased risk for a CYP3A4-related disorder.

In certain embodiment, the presence of a minor allele of the polymorphism is predictive of lower levels of CYP3A4 in target tissue and is associated with a decreased CYP3A4 mRNA expression.

In another aspect, there is provided herein, a kit comprising an assay for detecting the allelic status of one or more polymorphisms in a nucleic acid sample of a subject, wherein the polymorphism is one or more of: i) a CYP3A4-associated SNP (rs35599367 [SEQ ID NO: 152]) located in intron6 (C>T); or, ii) a SNP in linkage disequilibrium therewith.

In certain embodiment, the kit further includes instructions for correlating the assay results with the subject's risk for having or developing a CYP3A4-related disorder.

In certain embodiment, the kit further includes instructions for correlating the assay results with the subject's prognostic outcome for the disorder.

In certain embodiment, the kit further includes instructions for correlating the assay results with the probability of success or failure of a particular drug treatment in the subject.

In another aspect, there is provided herein, a method for finding a functional polymorphism in a target gene implicated in an CYP3A4-related disorder, comprising: i) providing a sample of a target tissue expressing the target gene; ii) measuring the target gene's allelic mRNA expression imbalance (AEI) by: a) quantitatively measuring the relative amounts of mRNA generated from each of two alleles in a transcribed region of the target gene, and b) comparing the mRNA expression of one allele against the other allele to obtain an AEI ratio; and, iii) using the AEI ratio as a phenotype to scan the target gene for regions containing polymorphisms, wherein a significant association between the AEI ratio and the polymorphism indicates that the polymorphism is a functional polymorphism that can serve as a biomarker for the CYP3A4-related disorder.

In certain embodiment, the polymorphism resides in an intronic region.

In certain embodiment, the polymorphism is a SNP.

In certain embodiment, the biomarker affects gene transcription, mRNA processing, mRNA splicing, or a combination thereof.

In certain embodiment, the target gene is a CYP3A4 gene locus.

In another aspect, there is provided herein, a method for determining metabolism of a statin drug comprising screening for an intron6 SNP.

In another aspect, there is provided herein, a method for determining metabolism of a statin drug comprising screening for a CYP3A4-associated SNP (rs35599367 [SEQ ID NO: 152]) located in intron6 (C>T).

In another aspect, there is provided herein a biomarker for detecting variability in CYP3A4 comprising intron6 SNP.

In another aspect, there is provided herein a biomarker for detecting variability in CYP3A4 comprising a CYP3A4-associated SNP (rs35599367 [SEQ ID NO: 152]) located in intron6 (C>T).

In another aspect, there is provided herein, a biomarker for determining a dosing requirement of a CYP3A4-metabolizing therapeutic agent comprising SNP (rs35599367 [SEQ ID NO: 152]) located in intron6 (C>T).

In another aspect, there is provided herein a biomarker for determining a response of a CYP3A4-metabolizing therapeutic agent comprising SNP (rs35599367 [SEQ ID NO: 152]) located in intron6 (C>T).

In another aspect, there is provided herein a biomarker for determining toxicity of a CYP3A4-metabolizing therapeutic agent comprising SNP (rs35599367 [SEQ ID NO: 152]) located in intron6 (C>T). In certain embodiment, the therapeutic agent is a statin drug. In certain embodiment, the therapeutic agent is an anti-cancer drug. In certain embodiment, the therapeutic agent is a drug having a narrowly defined dosage regimen.

In another aspect, there is provided herein a method of clinical pharmacogenomic screening comprising: a) screening a sample for the presence of at least one or more biomarkers described herein, where the presence of the one or more biomarkers is indicative of a patient with altered metabolism; and b) including a reference control in a random or predetermined manner in the screening, wherein the reference control comprises DNA comprising a biomarker indicative of a patient with altered metabolism, wherein the detection of the presence of one or more biomarkers in one or more drug-metabolizing genes in the reference control verifies that the screening is effective to detect the same one or more biomarkers in one or more drug-metabolizing genes in the sample.

In another aspect, there is provided herein a method of personalized medical therapy, comprising: i) performing the method of screening described herein on samples from a target patient population to identify patients with a genetic profile comprising one or more mutations in the CYP3A4 or other gene associated with drug metabolism; and ii) treating patients identified in step i) as possessing a particular genetic profile with a therapy of interest particular to the identified genetic profile.

In certain embodiments, the genetic profile is indicative of a patient with altered metabolism.

In certain embodiments, the altered metabolism is selected from the group consisting of: poor metabolizer, intermediate metabolizer, extensive metabolizer, and ultrarapid metabolizer.

In certain embodiments, the genetic profile is indicative of the effectiveness of the therapy of interest in the patient.

In certain embodiments, the genetic profile is indicative of a patient with a genetic disorder.

In certain embodiments, the genetic profile is indicative of a patient who should not be treated with a particular therapy.

In certain embodiments, the therapy of interest is used to treat a disease or disorder selected from the group consisting of: cancer, heart disease, neurological disorders, psychiatric disorders, autoimmune disorders, and metabolic disorders.

In certain embodiments, the one or more mutations comprises a mutation in CYP3A4 and wherein the therapy of interest comprises administration of at least one statin to the patient.

In another aspect, there is provided herein a method for identifying a cell that can be used to generate isolated genomic DNA suitable for use as a reference control, wherein the method comprises: a) prospectively screening a human volunteer for the presence of a human genomic DNA sequence comprising one or more polymorphisms in a nucleic acid sample of the volunteer associated with a genetic predisposition that determines a patient's predicted degree of response to a particular therapy; wherein the polymorphism is one or more of: i) CYP3A4-associated SNP (rs35599367 [SEQ ID NO: 152]) located in intron 6 (C>T); or, ii) a SNP in linkage disequilibrium therewith, wherein the allelic status of the polymorphism in the subject is predictive of the subject's risk for having or developing the CYP3A4-related disorder; b) isolating the cell from the volunteer that possesses the one or more mutations of interest; and c) subjecting DNA from the cell to a plurality of validated genomic screening assays.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file may contain at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

The invention can be more fully understood from the following detailed description, the drawings and the Sequence Descriptions that form a part of this application. The Sequence Descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 CFR §§1.821-1.825. The Sequence Descriptions contain the three letter codes for amino acids as defined in 37 CFR §§1.821-1.825, which are incorporated herein by reference.

FIG. **1**: Allelic mRNA/hnRNA expression ratios of CYP3A4 in human livers measured with a primer extension assay (SNaPshot) using multiple marker SNPs (FIG. **3**) (FIG. **1**A) or intron6 SNP rs35599367 [SEQ ID NO: 152] only (FIG. **1**B). Allelic RNA ratios were normalized to gDNA ratios set at 1. Data represent the average of 2-3 measurement using single or multiple marker SNPs. An arrow indicates samples with AEI ratios significantly different from 1 ($P<0.05$). All allelic RNA ratios in Panel B are significantly different from 1 (ANOVA with Dunnett post-test, $P<0.05$).

FIGS. **2**A-**2**B: Box plot of CYP3A4 mRNA levels (FIG. **2**A) and enzyme activity (FIG. **2**B) in human liver samples, grouped by intron6 SNP genotype.

FIG. **3**: Location and rs number of marker SNPs used for AEI measurements. Intron6 SNP rs35599367 [SEQ ID NO: 152] is in red.

FIG. **4**: Association between genotypes and allelic RNA expression imbalance (AEI). Only intron6 SNP rs35599367 [SEQ ID NO: 152] and much less strongly SNP rs2246709 associated with AEI with an adjusted $P<0.05$.

FIG. **5**: LD plot for 13 polymorphisms in CYP3A4. Detailed SNP information is provided in Table 1. SNP6 is intron6 SNP rs35599367 [SEQ ID NO: 152], only partial LD with SNP 7 rs2246709.

FIGS. **6**A-**6**D: Correlation between mRNA expression of four transcription factors and CYP3A4 in human livers.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will now be described with occasional reference to the specific embodiments of the invention. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The disclosure of all patents, patent applications (and any patents that issue thereon, as well as any corresponding published foreign patent applications), GenBank and other accession numbers and associated data, and publications mentioned throughout this description are hereby incorporated by reference herein. It is expressly not admitted, however, that any of the documents incorporated by reference herein teach or disclose the present invention.

The present invention may be understood more readily by reference to the following detailed description of the embodiments of the invention and the Examples included herein. However, before the present methods, compounds and compositions are disclosed and described, it is to be understood that this invention is not limited to specific methods, specific cell types, specific host cells or specific conditions, etc., as such may, of course, vary, and the numerous modifications and variations therein will be apparent to those skilled in the art. It is also to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting.

In a broad aspect, the inventors herein found common polymorphisms in CYP3A4 by measuring allelic hnRNA/mRNA expression in human autopsy livers. A detectable allelic RNA expression imbalance (AEI) is a direct measure of cis-acting regulatory factors in CYP3A4 that affect RNA expression, processing, or turnover.

The results described herein demonstrate that an intron6 SNP of CYP3A4 fully accounts for the observed allelic mRNA expression pattern and correlates with CYP3A4 enzyme activity in human livers, while previously suggested polymorphisms had no effect.

Moreover, intron6 SNP was significantly associated with stable statin dosage taken for cholesterol control and with achievement of therapeutic LDL goal in a cohort of CAD patients.

EXAMPLE I

Methods:

Tissue samples. 133 liver autopsy/biopsy samples were obtained from The Cooperative Human Tissue Network Midwestern and Western Division, under a protocol approved by the Ohio State University Institutional Review Board (OSU IRB).

Patients. Subjects were participants in the Ohio State University Coronary Artery Disease Study, with patients presenting to the OSU Heart Center with symptomatic cardiovascular disease requiring PCI calls. 275 patients documented to be taking stable doses of a statin for lipid control were selected. Enrollment and trial conditions have been approved by the OSU IRB, with written informed consent obtained from each patient. The study population reflects demographics of the Columbus area and surrounding rural counties of Ohio.

DNA and RNA preparation. Preparation of genomic DNA, RNA and cDNA from tissues or blood samples were performed as described (16-18).

Quantitative analysis of allelic ratios in genomic DNA and RNA using SNaPshot. The detailed method has been published (16,17). Briefly, a fragment of DNA or RNA (after conversion to cDNA) surrounding a marker SNP was PCR amplified, followed by a primer extension assay (SNaPshot) that targets the polymorphic site. Seven marker SNPs located in either 3'UTR or intronic regions were used to measure allelic ratios of mature mRNA (3'UTR markers) or hnRNA (intronic markers) in 73 out of the 133 livers heterozygous for at least one marker SNP. Genomic DNA (gDNA) allelic ratios, normalized to 1, served as internal control; none of the subjects displayed gDNA copy number variants, detectable by a significant deviation from unity. Deviations of allelic RNA ratios from 1 (after normalization to DNA ratios), i.e., allelic expression imbalance (AEI), indicates the presence of cis-acting polymorphisms in CYP3A4 that affect mRNA expression levels.

Genotyping: Thirteen SNPs in CYP3A4 (including the 7 marker SNPs) were genotyped in gDNA from liver samples with a multiplex SNaPshot assay (19) or allele specific real-time PCR (20), as shown in Table 1.

TABLE 1

Polymorphisms tested in liver samples.

SNP #	SNP ID	position
1	TGT ins	−11231
2	rs2737418 G > T	−7310
3	rs2740574 A > G (*1B)	−392
4	rs2687105 A > T	intron 2
5	rs28988579 T > G	intron 4
6	rs35599367 C > T	intron 6
7	rs2246709 C > T	intron 7
8	rs4646437 C > T	Intron 7
9	rs2242480G > A	intron10
10	rs3735451A > G	intron 12
11	rs28988604C > T	3'UTR
12	rs28969391delT	3'UTR
13	rs28371763A > T	3'UTR

Seven SNPs in CYP3A4/3A5 were genotyped in gDNA from 275 patients, as shown in Table 2.

TABLE 2

Polymorphisms tested in 275 patients.

SNP ID	Gene	Position
TGT ins	CYP3A4	Enhancer, −11231
rs2740574	CYP3A4	Promoter, −392, *1B
rs35599367	CYP3A4	Intron 6
rs4987161	CYP3A4	Exon 7 (F189S)
rs28371759	CYP3A4	Exon 10 (L293P)
rs776746	CYP3A5	Intron 3, *3
rs41303343	CYP3A5	Exon 11, *7

PCR conditions and primer sequences are shown in Table 3, Table 4, Table 5.

Quantitative mRNA analysis of CYP3A4 and transcription factors PXR, RXRa, CAR, and HNF4a in human livers: mRNA levels were measured with real-time PCR (16,21) using gene-specific primers (22) (Table 3, Table 4, Table 5) and SYBR Green (Applied Biosystems), with GADPH mRNA as an internal control.

Table 3: Genotyping or AEI assays. Column labeled "Sequence of PCR primers and assay condition" discloses SEQ ID NOS 1-42, respectively, in order of appearance and column labeled "Primer extension primers and condition" discloses SEQ ID NOS 43-56, respectively, in order of appearance.

TABLE 3

Genotyping or AEI assays

Snap shot assay	SNP#	rs#	location	Sequence of PCR primers and assay condition	Primer extension primers and condition
	2	rs2737418	7.3 kb upstream	F: GAACTTGCTGACCCTCTGCTTT [SEQ ID NO: 1] R: TAGCAAGCCACAGACAGCA [SEQ ID NO: 2] Choice Tag, 60° C. extension	CATTCTCCTTTAACCT GTTGACGA [SEQ ID NO: 43] 60° C. extension
	3	rs2740574	promoter	F: AGAGCCATGACAGGGAATAAGACT AGA [SEQ ID NO: 3] R: TGGGCTATGTGCATGGAGCTT [SEQ ID NO: 4] JumpStart RedTag, 60° C. extension	AGGACAGCCATAGAG ACAAGGGCA [SEQ ID NO:44] 55° C. extension
	5	rs28988579	intron 4	F: TTTCCTTTCCAATCTGTATGCC [SEQ ID NO: 5] R: AAAGAACTGAAGGCTTCCCTC [SEQ ID NO: 6] Choice Tag, 60° C. extension	AGTATAATGTTGAGT AAATGTGGTGAAT [SEQ ID NO: 45] 55° C. extension
	6	rs35599367	intron 6	F: CTGAAGCACAGTGCTTACCCAT [SEQ ID NO: 7] R: GGTGCCAGTGATGCAGCT [SEQ ID NO: 8] Choice Tag, 60° C. extension	CCAGTGATGCAGCTG GCCCTAC [SEQ ID NO: 46] 60° C. extension

TABLE 3-continued

Genotyping or AEI assays					
Snap shot assay	SNP#	rs#	location	Sequence of PCR primers and assay condition	Primer extension primers and condition
	8	rs4646437	intron7	TTATGATTTGGGTTATTCTAGGAG AC [SEQ ID NO: 9] CCTGGAGCAATTCTAGTTTTCTCT [SEQ ID NO: 10] Choice Tag, 60° C. extension	GGCAGGTCTATGCAT AAGGAGCACC [SEQ ID NO: 47]
	11	rs28988604	3UTR	F: TGGTCATTGTAATCACTGTTGGC [SEQ ID NO: 11] R: TTAAGTGTTCATTGCATCGAGAC [SEQ ID NO: 12] Choice Tag, 60° C. extension	(T15)CAAACTGCTAGG ATTACAGGC [SEQ ID NO: 48] 15 Ts were added at 5' end [SEQ ID NO: 49] 60° C. extension
	12	rs28969391	3'UTR	same as above	AAGCCTGGCCTACAT GGT [SEQ ID NO: 50] 60° C. extension
	13	rs28371763	3UTR	same as above	(T4)GTGAGAGTGAGA CTCAGTCTTAAAAA [SEQ ID NO: 51] 4 Ts were added at 5' end 60° C. extension
Note: SNP 11-13 can be multiplexed					
	7	rs2246709	intron 7	F: TTTAGCTATCAGCCCCCTGT [SEQ ID NO: 13] R: TGAAGCCAGCAGAAGAAAGAA [SEQ ID NO: 14] JumpStart RedTag, 60° C. extension	(T11)CAACCACTAATC AACTTTCTGC [SEQ ID NO: 52] 11 Ts were added at 5' end [SEQ ID NO: 53] 55° C. extension
	9	rs2242480	intron 10	F: GCTATGAAACCACGAGCAGTGT [SEQ ID NO: 15] R: GGGAAGTGGTGAGGAGGC [SEQ ID NO: 16]	(T18)CCTCCCTCCTTCT CCATGTA [SEQ ID NO: 54] 18 Ts were added at 5' end [SEQ ID NO: 55] 55° C. extension
	10	rs3735451	intron 12	F: ATAGATGATGAATGCTCTCACTG TCC [SEQ ID NO: 17] R: GGGATCTGCAACAGTTAAACAAG [SEQ ID NO: 18]	TTTTTTTGCCCATTAC TCCAT [SEQ ID NO: 56] 55° C. extension
Note: SNP 7, 9 and 10 can be multiplexed					
Allele specific PCR assay	1	TGT ins	11 kb upstream	common F: GCTCATAGAATCCTGGGCAT [SEQ ID NO: 19] TGTwtR: CCTATCTAGCCATTAGAACCACA TGT [SEQ ID NO: 20] TGTinsR: CCTATCTAGCCATTAGAACCACA TGTACA [SEQ ID NO: 21]	
	4	rs2687105	intron 2	snpF: GCTGCACCTTATGGGTGTGT [SEQ ID NO: 22] wtF: GCTGCACCTTATGGGTGTGA [SEQ ID NO: 23] common R: ATTGACCACCACTGTCTCATCTC [SEQ ID NO: 24]	

TABLE 3-continued

Genotyping or AEI assays					
Snap shot assay	SNP#	rs#	location	Sequence of PCR primers and assay condition	Primer extension primers and condition
	5	rs28988579	intron 4	wtF: GTATAATGTTGAGTAAATGTGGT GAGTT [SEQ ID NO: 25] snpF: GTATAATGTTGAGTAAAT-GTGGT GATTG [SEQ ID NO: 26] common R: AAAGAACTGAAGGCTTCCCTC [SEQ ID NO: 27]	
	6	rs35599367	intron 6	wtF: GTGTCTCCATCACACCCTGC [SEQ ID NO: 28] snpF: GTGTCTCCATCACACCCCGT [SEQ ID NO: 29] common R: GGTGTTATCAGGTGCCAGTG [SEQ ID NO: 30]	
Primers for real-time PCR to quantitate mRNA				primer sequence	
			CYP3A4	F: CTCTCATCCCAGACTTGGCCA [SEQ ID NO: 31] R: ACAGGCTGTTGACCATCATAAAAG [SEQ ID NO: 32]	
			PXR	F: CAAGCGGAAGAAAAGTGAACG [SEQ ID NO: 33] R: CACAGATCTTTCCGGACCTG [SEQ ID NO: 34]	
			RXR	F: GAGCGGCAGCGTGGCAAGG [SEQ ID NO: 35] R: GGCAAATGTTGGTGACAGGG [SEQ ID NO: 36]	
			HNF4a	F: ACATGGACATGGCCGACTAC [SEQ ID NO: 37] R: CTCGAGGCACCGTAGTGTTT [SEQ ID NO: 38]	
			CAR	F: CACATGGGCACCATGTTTGA [SEQ ID NO: 39] R: AAGGGCTGGTGATGGATGAA [SEQ ID NO: 40]	
			GADPH	F: ACTCCTCCACCTTTGACGCT [SEQ ID NO: 41] R: GGTCCACCACCCTGTTGC [SEQ ID NO: 42]	

Table 4: Multiplex PCR and Snapshot assay for CYP3A4 and CYP3A5 SNP genotyping. Column labeled "PCR primer sequence" discloses SEQ ID NOS 57-68, respectively, in order of appearance and column labeled "Primer extension primers" discloses SEQ ID NOS 69-75, respectively, in order of appearance.

TABLE 4

Multiplex PCR and Snapshot assay for CYP3A4 and CYP3A5 SNP genotyping			
SNP	PCR primer sequence	Primer extension primers	Final primer conc in snapshot reaction
TGT ins	F: GCCTGCATTTTATCTCTGTCTCGTGG [SEQ ID NO: 57] R: GAAGGTGGGAAACAGCCAGATCAGA [SEQ ID NO: 58]	(T8)ATTCCCTATCTAGCCAT TAGAACCACA [SEQ ID NO :69]	20 nM

TABLE 4-continued

Multiplex PCR and Snapshot assay for CYP3A4 and CYP3A5 SNP genotyping			
SNP	PCR primer sequence	Primer extension primers	Final primer conc in snapshot reaction
rs2740574	F: AGAGCCATGACAGGGAATAAGACTAGA [SEQ ID NO: 59] R: TGGGCTATGTGCATGGAGCTT [SEQ ID NO: 60]	(T15) AGGACAGCCATAGAGACAA GGGCA [SEQ ID NO: 70]	50 nM
rs35599367	F: CTGAAGCACAGTGCTTACCCAT [SEQ ID NO: 61] R: GCCACAACATAGTAAACGAAGAAGGGCA [SEQ ID NO: 62]	CCAGTGATGCAGCTGGCCCT AC [SEQ ID NO: 71]	80 nM
rs4987161	same as above	(T20) GTTGAGAGAGTCGATGTTC ACTCCA [SEQ ID NO: 72]	80 nM
rs28371759	F: CTGTGATGCCCTACATTGATCTGATTTA CCTA [SEQ ID NO: 63] R: CTGGGAAGTGGTGAGGAGGCATTTT [SEQ ID NO:64]	(T16)TCTCCTTTCAGCTCTGT CCGATC [SEQ ID NO: 73]	60 nM
rs776746	F: GTATGTACCACCCAGCTTAACGAATGCTC [SEQ ID NO: 65] R: CACACAGGAGCCACCCAAGGC [SEQ ID NO:66]	TGTGGTCCAAACAGGGAAG AGATA [SEQ ID NO: 74]	50 nM
rs1303343	F: CCAATTCTGTTTCTTTCCTTCCAGGCA [SEQ ID NO: 67] R: AACCAGCCTGGGTCAGGGTGAG [SEQ ID NO: 68]	(T2)CATCTGTACCACGGCAT CATAGGTA [SEQ ID NO: 75]	15 nM
	Readymix Taq, 60° C. extension Final primer cont for rs2740574 and rs35599367 is 300 nM, others are 150 nM CYP3A4 PCR and sequencing primers (ref sequence AF280107)	55° C. extension	

Table 5: Primers and sequencing primers. PCR primers disclosed as SEQ ID NOS 76-95, respectively, in order of appearance and Sequencing primers disclosed as SEQ ID NOS 96-151, respectively, in order of appearance.

TABLE 5

PCR Primers and Sequencing Primers		
PCR primers Amplified region	Sequence 5'-3'	Sequencing primers Sequence 5'-3'
Promoter 1(49947 to 51960)	F: TTACAATAGCAATGACCTGGAACCAATCC [SEQ ID NO: 76] R: GTGCAACCACAAACAATTAGGAACCTGT [SEQ ID NO: 77]	TTACAATAGCAATGACCTGGAACCA ATCC [SEQ ID NO: 96] AATGGCAGGCACTGGAATT [SEQ ID NO: 97] GTGCAACCACAAACAATTAGGAACC TGT [SEQ ID NO: 98]

TABLE 5-continued

PCR Primers and Sequencing Primers		
PCR primers Amplified region	Sequence 5'-3'	Sequencing primers Sequence 5'-3'
Promoter 2 (51740 to 53696)	F: AGCCCAGGAGGCAGCAGTTGC [SEQ ID NO: 78]	AGCCCAGGAGGCAGCAGTTGC [SEQ ID NO: 99]
	R: GTCTTCCTAAAGGAGTGACTGTTTGCA TTATCAT [SEQ ID NO: 78]	TATGAAGTGAAGGCCAGAAACGA [SEQ ID NO: 100]
		ACATGAAAAACAAAGCAACTCCAAC [SEQ ID NO: 101]
Promoter 3 (53408 to 55776)	F: TTGAGCCTGGGAGGCTGCG [SEQ ID NO: 80]	TTGAGCCTGGGAGGCTGCG [SEQ ID NO: 102]
	R: TAGCTTCTTCATTCGGTCTCAGTCCACTT [SEQ ID NO: 81]	ATGCTGGTTGCTGGTTTATTCTA [SEQ ID NO: 103]
		GAGTTGGGCATGATGCCTTT [SEQ ID NO: 104]
Promoter 4 (55681 to 59417)	F: TCATTTTTGTAGAGCCTGAGGAGTGTCCA [SEQ ID NO: 82] R: GATTTACCTGCCCTACAAACTTTAGGAG GTGG [SEQ ID NO: 83]	TCATTTTTGTAGAGCCTGAGGAGTGT CCA [SEQ ID NO: 105] CAAGAATGCTACCGGCACAA [SEQ ID NO: 106]
		TGAATCCTGGCTCTGCTAAAGC [SEQ ID NO: 107] ACCACTGGGAGCTTAAGTAAAGGG [SEQ ID NO: 108] TCAAGTTTTCCCCTACTGAGAAGAAT [SEQ ID NO: 109]
Promoter 5 (59230 to 62323)	F: GTTACCTTCTGTGGAATTAAGTGGCAGA ACT [SEQ ID NO: 84] R: GACAGAGTTTCACCATGTTAGCCAGGC [SEQ ID NO: 85]	GTTACCTTCTGTGGAATTAAGTGGCA GAACT [SEQ ID NO: 110]
		TTTTATCCCAGGGATTCCAG [SEQ ID NO: 111]
		GAAATTCATCCCAACAAGCCACACC [SEQ ID NO: 112] GACAGAGTTTCACCATGTTAGCCAGGC [SEQ ID NO: 113]
Promoter to intron 3 (61296 to 68200)	F: AGGGACCAGAGCCATGACAGGG [SEQ ID NO: 86]	AAGATGTTCAGGCCGGGC [SEQ ID NO: 114]
	R: TGACAAGAGCTTCATCCCAAGAGGC [SEQ ID NO: 87]	GTAGTGGAGGCTTCTCACATGTCA [SEQ ID NO: 115] GCAGCCCAGGAGTCAGAAAC [SEQ ID NO: 116] GGATCCATTTATACACACCATGCTT [SEQ ID NO: 117] GATTCAGACCATATCACTGGCACT [SEQ ID NO: 118] TCATTGCCGTCAGAGTTACTGTTATTA [SEQ ID NO: 119] CCTGCTTCAATCCTCTCCGA [SEQ ID NO: 120] GTGAAAACTCTGAGCAAGTGTTGTA ATT [SEQ ID NO: 121]

TABLE 5-continued

PCR Primers and Sequencing Primers

PCR primers Amplified region	Sequence 5'-3'	Sequencing primers Sequence 5'-3'
Intron 2 to intron 4 (667947 to 73642)	2F2: TTTCATTGGCTTCGACTGTTTTCATCC [SEQ ID NO: 88]	TTTCATTGGCTTCGACTGTTTTCATCC [SEQ ID NO: 122]
	2R2: ACAGGATGAAGTGGACGTGGAACCTT [SEQ ID NO: 89]	GGATCCCATGTGTCACCAGG [SEQ ID NO: 123]
		CCCGTGTCCATGTGTTCTCA [SEQ ID NO: 124] CTTCGTCAGATGGATAGATTGCAA [SEQ ID NO: 125] GTTCTTCCATTTGTTTGTGTCCTCT [SEQ ID NO: 125] TGCTGACTTGATCCTGGTGG [SEQ ID NO: 127] TCTTAGTTATTTCTTGTCTCCTGCTAGC [SEQ ID NO: 128] TCTTTGTAGGTCTCTAAGAACTTGCT TTAT [SEQ ID NO: 129]
Intron 3 to intron 8 (73332 to 79586)	3F2: ATTATGTAAAGTCAGGATCAAAGTCTGG CTTCC [SEQ ID NO: 90]	ATTATGTAAAGTCAGGATCAAAGTCT GGCTTCC [SEQ ID NO: 130]
	3R2: TCCCTTCTGAGAATATGGCTCCTTGAAG [SEQ ID NO: 91]	TGGTGCCATGTTTGAAAGTTCTT [SEQ ID NO: 131] ATGTGCTTTGATTTTGTGTGTTGAT [SEQ ID NO: 132] CCACGTGGATAATTTGCATGTAA [SEQ ID NO: 133] TTGAAAGAGTAAGTAGAAGCGCAGC [SEQ ID NO: 134] TGAGCAGGGAGTATAGAGAATAAGGAT [SEQ ID NO: 135] TCAATCTAGAGACCTCATACATTTTT AGCT [SEQ ID NO: 136] GAGAAAACTAGAATTGCTCCAGGTAAA [SEQ ID NO: 137]
Intron 8 to intron 11 (79395 to 84596)	4F: AAATGAAAGTCCCTATCAGGCCACCTG [SEQ ID NO: 92]	AAATGAAAGTCCCTATCAGGCCACCTG [SEQ ID NO: 138]
	4R: TCTTTCTCCCCACACCTCCATAGAATA [SEQ ID NO: 93]	TTGCAGCTACCTAATACATCTAACATCC [SEQ ID NO: 139]
		GCTAGTGCCATTGAAGATCAATTTTA [SEQ ID NO: 140] AATATTGGTCCCTTAAGTTCCCTCA [SEQ ID NO: 141] ACATGGAGAAGGAGGGAGGAG [SEQ ID NO: 142] GCTGACTCAGCTCTCCCCAC [SEQ ID NO: 143] ACCAGTATGAGTTAGTCTCTGGAGCTC [SEQ ID NO: 144]
Intron 11 to intron 13 (84264 to 89410)	5F: CATCCACAATTCCAACAACTTACGATGA AG [SEQ ID NO: 94]	CATCCACAATTCCAACAACTTACGAT GAAG [SEQ ID NO: 145]
	5R: CTATTTAGGCTCTGGCTGCTCTTGCAA [SEQ ID NO: 95]	ATTTCAATGACCAGCCCACAA [SEQ ID NO: 146]
		CATTGGAATCACCAGGGAGC [SEQ ID NO: 147] TTACTTCTCTGCTCTGTTATTGGATAC TG

TABLE 5-continued

PCR Primers and Sequencing Primers		
PCR primers Amplified region	Sequence 5'-3'	Sequencing primers Sequence 5'-3'
		[SEQ ID NO: 148] CTCCCTGGCAATTTTCTTGC
		[SEQ ID NO: 149] TTACAAAGCATTATTGTCATTACTGCAT
		[SEQ ID NO: 150] CTGTGACTTTGCCCATTGTTTAGA
		[SEQ ID NO: 151]

Sequencing CYP3A4: The region from ~10,000 bp upstream of transcription start site to the last exon (from 50013 to 89410 in AF280107, total length 39,397 bp) was sequenced in two liver samples that showed allele-specific RNA expression. PCR and sequencing primers are shown in Table 10.

CYP3A4 enzymatic activity assay: CYP3A4 activities were quantified from liver microsomes with testosterone as a probe as described (23).

Data analysis: Association between discrete variables and genotypes were analyzed with Helix-Tree software (Golden Helix, Bozeman Mont.). LD plots were generated using Haploview. Multiple linear regression analysis was used for testing genotype effects on RNA expression, enzyme activity, and statin dose requirement using SPSS or Minitab software.

Results:

Scanning for cis-acting CYP3A4 polymorphisms that affect mRNA levels:

Allelic mRNA expression of CYP3A4 was measured in human livers using 3 frequent 3' UTR marker SNPs (FIG. **3**). Because of high CYP3A4 expression, 4 intronic SNPs also served to measure allelic expression of CYP3A4 hnRNA (24). Among 133 liver samples screened, 73 were heterozygous for at least one of the seven marker SNPs and therefore suitable for AEI measurement. Of the 73 samples, 7 were identified showing strong allelic expression imbalance (AEI), with the main allele expressed less than the minor allele (0.2 to 0.5 times), observed with both intronic and exonic marker SNPs (FIG. **1**A). Therefore, a cis-acting polymorphism(s) in CYP3A4 affects both mRNA and hnRNA levels equally.

To search for responsible polymorphism(s), 13 CYP3A4 polymorphisms (Table 1) were genotyped and the association between allelic RNA ratios and genotype tested.

A single SNP (rs35599367 [SEQ ID NO: 152]) located in intron6 (C>T) showed highly significant association with AEI (adjusted p value $9.12\times)10^{-10}$ (FIG. **4**), while another SNP rs2246709 also scored with moderate significance (P=0.034), a likely result of partial linkage disequilibrium (LD) with the intron6 SNP (FIG. **5**).

Other SNPs including previously identified promoter SNP rs2740574, TGT insertion, rs2737418, and rs4646437 did not show significant association (P>0.05). These results indicated that intron6 SNP is functional or in highly LD with a functional SNP.

To test this further, intron6 SNP was used as a marker, and AEI was measured in 10 heterozygous samples, including 3 heterozygous only for intron6 SNP. All 10 samples showed AEI with allelic ratios (minor T allele/major C allele) ranging from 0.16 to 0.62 (FIG. **1**B), with no differences between males and females (P=0.314). Therefore, the minor T allele of intron6 SNP is linked to reduced mRNA/hnRNA levels (the inverse allelic mRNA ratio of major C/minor T ranges from 1.6 to 6.25). The minor T allele is exclusively linked to the main CYP3A4 haplotype, except for low LD with rs2246709 (Table 6; LD plot FIG. **5**).

Table 6: Haplotype structure and estimated frequency of 13 SNPs tested in livers. Detailed SNP information is provided in Table 1. In haplotype 5, the minor T allele of intron6 SNP (#6 from the left) is exclusively linked to the major haplotype 1 (in bold), whereas it has no detectable LD with any of the other SNPs.

TABLE 6

Haplotype		EM Frequency
1	**D, G, A, A, T, C, T, C, G, A, C, T, A**	0.408
2	D, G, A, A, T, C, C, **C**, G, A, C, T, A	0.208
3	D, G, G, T, T, **C**, T, T, A, G, C, G, A	0.072
4	D, G, A, A, T, **C**, T, T, A, G, C, G, A	0.048
5	**D, G, A, A, T, T, C, C, G, A, C, T, A**	0.044
6	D, G, A, A, T, **C**, C, T, A, G, C, G, A	0.032
7	I, G, A, A, G, **C**, T, C, G, A, C, T, A	0.027
8	D, G, A, A, T, **C**, T, T, G, A, C, T, T	0.026
9	D, T, G, T, T, **C**, C, T, A, G, C, G, A	0.026
10	D, G, A, A, T, **C**, T, C, G, G, T, G, A	0.014

Sequencing of the entire CYP3A4 locus in two AEI-positive samples did not implicate any other polymorphisms that would have to be heterozygous in both samples, indicating that intron6 SNP is functional.

Intron6 SNP associates with decreased CYP3A4 mRNA level and enzyme activity in human livers:

Total CYP3A4 mRNA levels were measured in 93 liver samples. While mRNA levels did not differ between Caucasians and African Americans, females had −1.3 fold higher levels than males (95% CL 1.00-1.68, two-sided P=0.042) as reported (25). Livers with the main CC genotype of intron6 SNP had 1.71-fold (95% confidence interval (CI) 1.06-2.76) higher levels than CT/TT carriers (t test, two-sided p=0.028), with no interactions between genotypes and sex. To test the effect of CYP3A4 transcription factors (26-29), mRNA levels were also measured for pregnane X receptor

(PXR, NR112), constitutive androstane receptor (CAR, NR113), retinoid receptor (RXRa), and hepatocyte nuclear factor (HNF4α1A). CYP3A4 mRNA expression positively correlated with all four transcription factors, as reported (26-29) (FIGS. **6**A-**6**D).

After adjusting for age and transcription factors, intron6 SNP remained significantly linked to CYP3A4 expression (1.67-fold CC over CT/TT (95%, CI 1.11-2.46, p=0.014) (FIG. **2**A), showing that the genotype effect is independent of the transcription factors.

CYP3A4 enzyme activity (testosterone 6β-hydroxylation) in 23 livers was 2.46 fold higher for intron6 SNP CC than CT carriers, after adjusting for age (<15 child, >15 adult), sex, and use of inducers (phenobarbital, carbamazepine, nifedipine and dexamethasone) (two-sided p=0.037, 95% CI 1.07-5.62) (FIG. **2**B).

Consistent with allelic mRNA expression, these results demonstrate that intron6 SNP decreases CYP3A4 mRNA and protein levels in vivo. In contrast, CYP3A4*1B, TGT insertion, rs2737418, and rs4646437 had no effect on total CYP3A4 mRNA level or enzyme activity (P>0.05).

Intron6 SNP (C>T) associates with statin dose requirement and lipid control outcome:

The in vivo effect of intron6 SNP was assessed as the dosage of CYP3A4-metabolized statins required for reaching a pre-determined LDL goal. Intron6 SNP was genotyped in 275 patients on stable doses of atorvastatin, lovastatin, and simvastatin, or the non-CYP3A4 substrates rosuvastatin and pravastatin. Additional SNPs in CYP3A4 (*1B rs2740574, TGT insertion, *17 rs4987161, and *18 rs28371759) and CYP3A5 (*3 rs776746 and *5 rs41303343) (Table 5) were also genotyped. Three SNPs deviated from Hardy-Weinberg equilibrium (HWE), because of different allele frequency in Caucasian and African American populations, in which all SNPs followed HWE when analyzed separately (Table 7).

TABLE 7

SNPs tested in 275 patients.

			All Patients n = 275		White n = 240		Black and others n = 35	
SNP	Alleles	Location	MAF	HWE P	MAF	HWE P	MAF	HWE P
TGT	TGT Del/Ins	CYP3A4 enhancer	0.016	0.78	0.017	0.79	0.018	0.92
rs2740574	A > G	CYP3A4 promoter	0.105	6.06E−07	0.054	0.71	0.350	0.77
rs35599367	C > T	CYP3A4 intron 6	0.047	0.061	0.052	0.08	0	NA
rs4987161	T > C	CYP3A4 exon 7	0	NA	0	NA	0	NA
rs28371759	T > C	CYP3A4 exon 10	0	NA	0	NA	0	NA
rs776746	G > A	CYP3A5 intron 3	0.138	2.81E−08	0.088	0.09	0.376	0.28
rs1303343	T Del/Ins	CYP3A5 exon 11	0.012	4.53E−06	0.008	0.95	0.055	0.76

The allele frequency of intron6 SNP is ~5%, consistent with that reported in NCBI database. Absence of CYP3A4 SNPs *17 and *18 is consistent with reported low allele frequency.

A subset of 237 patients were on CYP3A4-metabolized statins (atorvastatin, lovastatin, simvastatin) with doses ranging from 5 mg to 80 mg. The statins were either evaluated together, or each separately to account for potency differences. Patients on all statins were divided into low-dose (<20 mg, mean 16±5 mg) and high-dose (>40 mg, mean 53±19 mg) (P<0.001) groups. Baseline characteristics and lipid levels did not differ between low-and high-dose groups, except for maximum triglyceride and stable HDL levels (P<0.05) (Table 8). Table 8 shows the baseline characteristics of patients on CYP3A4-metabolized statins (atorvastatin, lovastatin and simvastatin).

TABLE 8

Baseline characteristics of patients on CYP3A4-metabolized statins (atorvastatin, lovastatin and simvastatin).

	All patient n = 237	Low dose n = 121	High dose n = 116	P value
Dose (mg)	34 ± 23	16 ± 5	53 ± 19	<0.001
Age	62 ± 11	63 ± 13	62 ± 10	0.444
Maximum LDL (mg/dl)	105 ± 49	102 ± 52	108 ± 45	0.362
Stable LDL (mg/dl)	82 ± 33	85 ± 39	80 ± 24	0.297
Minimum HDL (mg/dl)	33 ± 10	34 ± 10	32 ± 9	0.141
Stable HDL (mg/dl)	35 ± 10	36 ± 10	33 ± 9	0.042*
Max Triglyceride (mg/dl)	256 ± 430	196 ± 159	318 ± 590	0.043*
Stable Triglyceride (mg/dl)	156 ± 106	145 ± 108	166 ± 104	0.159
Max total Cholesterol (mg/dl)	185 ± 79	175 ± 65	194 ± 91	0.085
Stable Cholesterol (mg/dl)	148 ± 41	149 ± 47	147 ± 35	0.650
Male	156/232 (67%)	74/120 (62%)	82/112 (73%)	0.070
White	204/228 (89%)	108/120 (90%)	97/108 (89%)	0.607

The minor intron6 SNP T allele was significantly associated with a lower stable statin dose (odd ratios=3.80, P=0.005). None of the other SNPs were significant. Table 9 shows the association of CYP3A4/3A5 SNP genotypes with statins dose requirement and cholesterol control outcome. *P<0.05, **P<0.01.

TABLE 9

Association of CYP3A4/3A5 SNP genotypes with statins dose requirement and cholesterol control outcome.

	Low dose vs high dose		Goal vs not goal	
SNP	Minor allele odd ratio (95% CL)	P value	Minor allele odd ratio (95% CL)	P value
TGT	0.31 (0.06~1.54)	0.165	1.24 (0.24~6.50)	0.794
rs2740574 (*1B)	1.30 (0.74~2.32)	0.388	0.58 (0.32~1.05)	0.083
rs35599367 (intron 6)	3.80 (1.38~10.35)	0.005**	3.49 (1.02~11.96)	0.039*
rs776746	0.96 (0.57~1.63)	0.902	0.65 (0.37~1.14)	0.139
rs41303343	0.94 (0.18~4.71)	0.942	0.24 (0.04~1.34)	0.097

*P < 0.05,
**P < 0.01.

Multiple linear regression analysis shows that the stable statin dose for intron6 T allele carriers was only 0.27 that for non T-carriers (P=0.019), after adjusting for maximum lipid levels (Table 10). Similar results were obtained when analyzing patients on atorvastain and simvastatin separately (Table 10). Therefore, intron6 SNP is linked to reduced statin dose requirement.

TABLE 10

CYP3A4 intron6 SNP genotypes and stable statin doses.

Statins	N	Dose ratio (T carrier/ non-Tcarrier)	95% confidence interval	P value
CYP3A4 substrates	237	0.27	0.19~0.66	0.019*
Atorvastatin	143	0.22	0.14~0.54	0.024*
Simvastatin	85	0.6	0.37~0.97	0.042*

*P < 0.05

For testing the association of intron6 SNP with cholesterol control outcome, 198 patients with available LDL data were divided into those who reached the cholesterol goal (LDL cholesterol<70 mg/dl; or 71-99 mg/dl with low/moderate risk; or 100-129 mg/dl with low risk) and those not at goal (>130 mg/dl; or 100-129 mg/dl with moderate/high risk; or 71-99 mg/dl with high risk) according to Adult Treatment Panel III Guidelines (30). Individuals bearing the T allele of intron6 SNP had a 3.5 fold higher chance of reaching goal cholesterol control than non T-carriers (P=0.039) (Table 2). In contrast, other SNPs did not show such associations.

Discussion of EXAMPLE I:

Example I shows that intron6 SNP rs35599367 [SEQ ID NO: 152] is significantly linked to reduced CYP3A4 mRNA expression and enzyme activity in human livers, and moreover, fully accounts for differences in allelic mRNA expression.

Since intron6 SNP is embedded in the main haplotype of CYP3A4, lacking substantial LD to any other SNPs, it had escaped detection by association studies using haplotype tag SNPs (14,15). None of the previously reported CYP3A4 SNPs, including promoter *1B, enhancer TGT insertion, enhancer rs2737418, and intron7 SNP rs4646437 had detectable effects on allelic mRNA expression, mRNA, and enzyme activity, arguing against a contribution of these SNPs to CYP3A4 variability.

The allele frequency of intron6 SNP in the examined cohort was ~5%, resulting in ~10% heterozygocity. Previously reported allele frequencies (35) were 0.043, 0.043, and 0.083, for African Americans, Chinese, and Caucasians, respectively, indicating that intron6 SNP allele frequency ranges from 4 to 8% in various ethnic groups.

A common molecular mechanism for an intronic SNP to alter mRNA levels is to affect RNA expression, elongation, splicing, or maturation. Since the allelic ratios were similar for mRNA and hnRNA in livers heterozygous for both exonic and intronic marker SNPs, splicing and mRNA turnover can be ruled out as main mechanisms. Moreover, CYP3A4 mRNA and hnRNA levels were shown to vary in parallel in human livers (36), arguing for an early event in expression and processing. For example, intron6 SNP could affect the folding of nascent RNA and hence elongation.

In silico RNA folding analysis shows that intron6 SNP promotes a loop-to-stem structural change (not shown), possibly impeding the binding of regulatory proteins.

Consistent with reduced expression of the minor allele, intron6 SNP was significantly associated with reduced stable dose requirements of statin drugs that are mainly metabolized by CYP3A4 (atorvastatin, lovastatin and simvastatin). Since statin doses are titrated to reach a desired LDL, this can be achieved at lower doses in carriers of the intron6 SNP T allele conveying reduced metabolism. In support of the inventors' belief, pharmacokinetics studies showed that inhibition of CYP3A4 activity drastically increased plasma concentrations of simvastatin and lovastatin (31,32), suggesting CYP3A4 activity is a major determinant of serum concentration of CYP3A4 metabolized statins.

The finding that patients carrying the intron6 T allele are also more likely to reach LDL goal may be related to insufficient dosage titration in subjects with normal CYP3A4 metabolism, or with lower fluctuations in statin levels between doses in T allele carriers. The previously identified CYP3A4 promoter SNP (*1B, −392 A>G) did not associate with lipid-lowering efficacy and safety of simvastin treatment (33), consistent with the results here showing this promoter SNP has no effect on hepatic mRNA expression. Further, the current results indicate that SNPs in CYP3A5 do not affect statin dose or treatment outcomes, consistent with reports that CYP3A5 does not play a major role in statin metabolism (34). Therefore, intron6 SNP is the only CYP3A polymorphism shown to affect statin therapy.

Even though CYP3A4 activity shows considerable interindividual variability, new drugs are often targeted for metabolism by CYP3A4, to avoid problems arising from null mutations in other drug metabolizing CYP enzymes, such as CYP2D6.

The results presented here show that a portion of the variability in CYP3A4 can be accounted for by intron6 SNP. The clinical relevance of this finding is demonstrated by the impact of intron6 SNP on the titrated dose of two statin drugs that depend on CYP3A4 for their elimination. Because CYP3A4 is involved in the metabolism of approximately half of all clinically used drugs, the intron6 SNP is now believed by the inventors herein to affect dosing requirements, response, and toxicity of numerous drugs, including anticancer agents with narrowly defined dosage regimens.

Therefore, CYP3A4 intron6 SNP is a valuable biomarker in clinical practice, and in drug discovery and development.

EXAMPLE II

The DNA isolated from cells can be used as positive human genomic reference controls (i.e., they have mutations present) or negative controls (i.e., they represent the normal or wild-type), in particular, for human CYP3A4 genes. This can ensure accurate and reliable clinical diagnostic testing for these genes.

The reference controls can be used, for example, in genotyping assays performed during clinical trials. Where the reference controls include a genetic variation typical of a patient who does not respond to therapy, the use of reference controls helps ensure that the genotyping assay used performs reliably such that non-responders are properly identified and data regarding the ineffectiveness of the investigative therapy for non-responders is properly identified. Similarly, where the reference controls include a genetic variation typical of a patient who metabolizes drugs at a different rate than normal patients (i.e., patients with mutant cytochrome P450 genes), the use of reference controls helps ensure the validity of the genetic variation so that these patients are properly identified and properly dosed and adverse drug reactions or ineffective therapies are avoided.

The reference controls can also be used in patient care. As with their use in clinical trials, it is essential that effective therapy is identified in a time-sensitive manner, so that the patient's condition is not worsened before appropriate therapy is initiated. It is also essential that appropriate dosing regimens are selected.

Accordingly, one aspect of the present invention relates to a method of testing a plurality of patients for their genetic predisposition to respond to a particular therapy. In this aspect, one or more reference controls are tested as "samples"—with known expected results. These controls can include a genetic variant associated with patients who show a predisposition to not respond to the therapy (positive controls) or can include a normal/wild type variant (negative controls), associated with patients who show a predisposition to respond to the therapy.

Thus, the reference controls can be used by testing laboratories to ensure that their diagnostics assays are performing correctly and identify the genetic variations that convey resistance to drug therapy or reduced metabolic state. To ensure that non-responders are properly identified, testing laboratories can include reference controls in each assay to determine the validity of the assay, and hence, patient results. The reference controls can be used at random, or at pre-determined intervals. In the same respect, testing laboratories can use the reference controls as panels to evaluate the accuracy of their laboratory staff.

Accordingly, another aspect of the present invention relates to a method of testing a plurality of patients for their genetic predisposition to show rapid or slowed metabolism, so that proper dosing regimens can be set. In this aspect, one or more samples that are tested are reference controls that include a genetic variant associated with patients having a predisposition to be "rapid" or "slow" metabolizers.

These polymorphisms are expressed in a number of phenotypes in the population such as, for example, a "poor" metabolizer, an "intermediate" metabolizer, an "extensive" metabolizer, and an "ultra-rapid" metabolizer. In certain situations, the "extensive" metabolizers can have at least one, and no more than two, normal functional alleles; the "intermediate" metabolizers can possess one reduced activity allele and one null allele; and, the "poor" metabolizers can carry two mutant alleles which result in complete loss of enzyme activity. In certain situations, the ultra-rapid metabolizers can carry multiple copies of functional alleles, and thus produce excess enzymatic activity. Thus, when certain drugs are administered, a "poor" metabolizer may not obtain a significant benefit from the drugs, but rather, experience exaggerated drug response and side effects when they receive standard doses. That is, if a metabolite is the active therapeutic moiety, "poor" metabolizer may show no therapeutic response. On the other hand, an "ultra-rapid" metabolizer may fail to respond to standard doses.

Various methods for pre-screening samples may be used. For example, biological samples can be pre-screened to ensure that they have the mutation of interest. Patient populations can be pre-screened, based on a variety of factors, to minimize the sample size needed to identify individuals that include the mutation. After performing an initial genomic screening on the samples to identify one or more samples which include the mutation of interest, the patients with these mutations can optionally be recalled to obtain additional biological material. This material can optionally be thoroughly sequenced to confirm the presence of the mutation of interest. The biological material can be immortalized, so it can provide a steady, on-demand source of the reference controls, or, alternatively, the cells themselves can be the reference controls. Various types of biological samples can be used, such as, but not limited to human genomic DNA present in any nucleic acid-containing sample of tissues, bodily fluids (for example, blood, serum, plasma, saliva, urine, tears, semen, vaginal secretions, lymph fluid, cerebrospinal fluid or mucosa secretions), individual cells or extracts of the such sources that contain the nucleic acid of the same, and subcellular structures such as mitochondria or chloroplasts, using protocols well established within the art.

In certain embodiments, the nucleic acid has been obtained from a human to be pre-screened for the presence of one or more genetic sequences that can be diagnostic for, or predispose the subject to, a medical condition or disease. As an alternative to prescreening patient populations, one can screen cell cultures commonly available for the genetic sequence of interest, and then expand and preserve the cell clones for future continuous supply

There are a number of known genomic assay methods for which the reference control can be used in testing where most involve hybridizing a primer with a DNA sample that may or may not include a SNP of interest. A diagnostic primer and/or probe can be tagged to permit rapid identification. Once hybridization has occurred, the DNA can be amplified, and the tagged primer and/or probe are detected. The validated primers can be used to confirm the validity of reference controls. Once the reference controls are validated, they can be used in commercially available assays as a reference control, and can be used to validate primers that are designed for use in these or other assays to determine the presence or absence of a particular mutation. Thus, while exemplary assay methods are described herein, the invention is not so limited.

While the invention has been described with reference to various and preferred embodiments, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted for elements thereof without departing from the essential scope of the invention. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from the essential scope thereof. Therefore, it is intended that the invention not be limited to the particular embodiment disclosed herein contemplated for carrying out this invention, but that the invention will include all embodiments falling within the scope of the claims.

The citation of any reference herein is not an admission that such reference is available as prior art to the instant invention. Any publications mentioned in this specification are herein incorporated by reference. Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

References

1. Danielson, P. B. (2002) *Curr Drug Metab* 3, 561-597.
2. Ozdemir, V., Kalow, W., Tang, B. K., Paterson, A. D., Walker, S. E., Endrenyi, L., and Kashuba, A. D. (2000) *Pharmacogenetics* 10, 373-388.
3. Rebbeck, T. R., Jaffe, J. M., Walker, A. H., Wein, A. J., and Malkowicz, S. B. (1998) *J Natl Cancer Inst* 90, 1225-1229.
4. Westlind, A., Lofberg, L., Tindberg, N., Andersson, T. B., and Ingelman-Sundberg, M. (1999) *Biochem Biophys Res Commun* 259, 201-205.
5. Wojnowski, L., and Kamdem, L. K. (2006) *Expert Opin Drug Metab Toxicol* 2, 171-182.
6. Lamba, J. K., Lin, Y. S., Thummel, K., Daly, A., Watkins, P. B., Strom, S., Zhang, J., and Schuetz, E. G. (2002) *Pharmacogenetics* 12, 121-132.
7. Garcia-Martin, E., Martinez, C., Pizarro, R. M., Garcia-Gamito, F. J., Gullsten, H., Raunio, H., and Agundez, J. A. (2002) *Clin Pharmacol Ther* 71, 196-204.
8. Amirimani, B., Walker, A. H., Weber, B. L., and Rebbeck, T. R. (1999) *J Natl Cancer Inst* 91, 1588-1590.
9. Spurdle, A. B., Goodwin, B., Hodgson, E., Hopper, J. L., Chen, X., Purdie, D. M., McCredie, M. R., Giles, G. G., Chenevix-Trench, G., and Liddle, C. (2002) *Pharmacogenetics* 12, 355-366.
10. Ball, S. E., Scatina, J., Kao, J., Ferron, G. M., Fruncillo, R., Mayer, P., Weinryb, I., Guida, M., Hopkins, P. J., Warner, N., and Hall, J. (1999) *Clin Pharmacol Ther* 66, 288-294.
11. Zeigler-Johnson, C., Friebel, T., Walker, A. H., Wang, Y., Spangler, E., Panossian, S., Patacsil, M., Aplenc, R., Wein, A. J., Malkowicz, S. B., and Rebbeck, T. R. (2004) *Cancer Res* 64, 8461-8467.
12. Kuehl, P., Zhang, J., Lin, Y., Lamba, J., Assem, M., Schuetz, J., Watkins, P. B., Daly, A., Wrighton, S. A., Hall, S. D., Maurel, P., Relling, M., Brimer, C., Yasuda, K., Venkataramanan, R., Strom, S., Thummel, K., Boguski, M. S., and Schuetz, E. (2001) *Nat Genet* 27, 383-391.
13. Matsumura, K., Saito, T., Takahashi, Y., Ozeki, T., Kiyotani, K., Fujieda, M., Yamazaki, H., Kunitoh, H., and Kamataki, T. (2004) *Mol Pharmacol* 65, 326-334.
14. Perera, M. A., Thirumaran, R. K., Cox, N. J., Hanauer, S., Das, S., Brimer-Cline, C., Lamba, V., Schuetz, E. G., Ratain, M. J., and Di Rienzo, A. (2008) *Pharmacogenomics J.*
15. Schirmer, M., Rosenberger, A., Klein, K., Kulle, B., Toliat, M. R., Nurnberg, P., Zanger, U. M., and Wojnowski, L. (2007) *Pharmacogenomics* 8, 443-453.
16. Wang, D., Johnson, A. D., Papp, A. C., Kroetz, D. L., and Sadee, W. (2005) *Pharmacogenet Genomics* 15, 693-704.
17. Pinsonneault, J., Nielsen, C. U., and Sadee, W. (2004) *J Pharmacol Exp Ther* 311, 1088-1096.
18. Miller, S. A., Dykes, D. D., and Polesky, H. F. (1988) *Nucleic Acids Res* 16, 1215
19. Dai, Z., Papp, A. C., Wang, D., Hampel, H., and Sadee, W. (2008) *BMC Med Genomics* 1, 24.
20. Papp, A. C., Pinsonneault, J. K., Cooke, G., and Sadee, W. (2003) *Biotechniques* 34, 1068-1072.
21. Wang, D., Papp, A. C., Binkley, P. F., Johnson, J. A., and Sadee, W. (2006) *Pharmacogenet Genomics* 16, 735-745.
22. Leeder, J. S., Gaedigk, R., Marcucci, K. A., Gaedigk, A., Vyhlidal, C. A., Schindel, B. P., and Pearce, R. E. (2005) *J Pharmacol Exp Ther* 314, 626-635.
23. Kolwankar, D., Vuppalanchi, R., Ethell, B., Jones, D. R., Wrighton, S. A., Hall, S. D., and Chalasani, N. (2007) *Clin Gastroenterol Hepatol* 5, 388-393.
24. Hirota, T., Ieiri, I., Takane, H., Maegawa, S., Hosokawa, M., Kobayashi, K., Chiba, K., Nanba, E., Oshimura, M., Sato, T., Higuchi, S., and Otsubo, K. (2004) *Hum Mol Genet* 13, 2959-2969.
25. Wolbold, R., Klein, K., Burk, O., Nussler, A. K., Neuhaus, P., Eichelbaum, M., Schwab, M., and Zanger, U. M. (2003) *Hepatology* 38, 978-988.
26. Vyhlidal, C. A., Gaedigk, R., and Leeder, J. S. (2006) *Drug Metab Dispos* 34, 131-137.
27. Goodwin, B., Redinbo, M. R., and Kliewer, S. A. (2002) *Annu Rev Pharmacol Toxicol* 42, 1-23.
28. He, P., Court, M. H., Greenblatt, D. J., and von Moltke, L. L. (2006) *J Clin Pharmacol* 46, 1356-1369.
29. Lamba, J., Lamba, V., and Schuetz, E. (2005) *Curr Drug Metab* 6, 369-383.
30. Grundy, S. M., Cleeman, J. I., Merz, C. N., Brewer, H. B., Jr., Clark, L. T., Hunninghake, D. B., Pasternak, R. C., Smith, S. C., Jr., and Stone, N. J. (2004) *J Am Coll Cardiol* 44, 720-732.
31. Neuvonen, P. J., Kantola, T., and Kivisto, K. T. (1998) *Clin Pharmacol Ther* 63, 332-341.
32. Jalava, K. M., Olkkola, K. T., and Neuvonen, P. J. (1997) *Clin Pharmacol Ther* 61, 410-415.
33. Fiegenbaum, M., da Silveira, F. R., Van der Sand, C. R., Van der Sand, L. C., Ferreira, M. E., Pires, R. C., and Hutz, M. H. (2005) *Clin Pharmacol Ther* 78, 551-558.
34. Park, J. E., Kim, K. B., Bae, S. K., Moon, B. S., Liu, K. H., and Shin, J. G. (2008) *Xenobiotica* 38, 1240-1251.
35. Thompson, E. E., Kuttab-Boulos, H., Witonsky, D., Yang, L., Roe, B. A., and Di Rienzo, A. (2004) *Am J Hum Genet* 75, 1059-1069.
36. Rodriguez-Antona, C., Sayi, J. G., Gustafsson, L. L., Bertilsson, L., and Ingelman-Sundberg, M. (2005) *Biochem Biophys Res Commun* 338, 299-305.

SEQUENCE LISTING

```
<160> NUMBER OF SEQ ID NOS: 152

<210> SEQ ID NO 1
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
```

primer

<400> SEQUENCE: 1

gaacttgctg accctctgct tt 22

<210> SEQ ID NO 2
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 2

tagcaagcca cagacagca 19

<210> SEQ ID NO 3
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 3

agagccatga cagggaataa gactaga 27

<210> SEQ ID NO 4
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 4

tgggctatgt gcatggagct t 21

<210> SEQ ID NO 5
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 5

tttcctttcc aatctgtatg cc 22

<210> SEQ ID NO 6
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 6

aaagaactga aggcttccct c 21

<210> SEQ ID NO 7
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 7

ctgaagcaca gtgcttaccc at 22

<210> SEQ ID NO 8
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 8

ggtgccagtg atgcagct 18

<210> SEQ ID NO 9
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 9

ttatgatttg ggttattcta ggagac 26

<210> SEQ ID NO 10
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 10

cctggagcaa ttctagtttt ctct 24

<210> SEQ ID NO 11
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 11

tggtcattgt aatcactgtt ggc 23

<210> SEQ ID NO 12
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 12

ttaagtgttc attgcatcga gac 23

<210> SEQ ID NO 13
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 13

tttagctatc agccccctgt 20

<210> SEQ ID NO 14
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 14

tgaagccagc agaagaaaga a 21

<210> SEQ ID NO 15
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 15

gctatgaaac cacgagcagt gt 22

<210> SEQ ID NO 16
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 16

gggaagtggt gaggaggc 18

<210> SEQ ID NO 17
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 17

atagatgatg aatgctctca ctgtcc 26

<210> SEQ ID NO 18
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 18

gggatctgca acagttaaac aag 23

<210> SEQ ID NO 19
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 19

gctcatagaa tcctgggcat 20

<210> SEQ ID NO 20
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 20

cctatctagc cattagaacc acatgt 26

<210> SEQ ID NO 21
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 21

cctatctagc cattagaacc acatgtaca 29

<210> SEQ ID NO 22
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 22

gctgcacctt atgggtgtgt 20

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 23

gctgcacctt atgggtgtga 20

<210> SEQ ID NO 24
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 24

attgaccacc actgtctcat ctc 23

<210> SEQ ID NO 25
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 25

```
gtataatgtt gagtaaatgt ggtgagtt                                    28


<210> SEQ ID NO 26
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 26

gtataatgtt gagtaaatgt ggtgattg                                    28


<210> SEQ ID NO 27
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 27

aaagaactga aggcttccct c                                           21


<210> SEQ ID NO 28
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 28

gtgtctccat cacaccctgc                                             20


<210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 29

gtgtctccat cacaccccgt                                             20


<210> SEQ ID NO 30
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 30

ggtgttatca ggtgccagtg                                             20


<210> SEQ ID NO 31
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 31

ctctcatccc agacttggcc a                                           21
```

<210> SEQ ID NO 32
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 32

acaggctgtt gaccatcata aaag 24

<210> SEQ ID NO 33
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 33

caagcggaag aaaagtgaac g 21

<210> SEQ ID NO 34
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 34

cacagatctt tccggacctg 20

<210> SEQ ID NO 35
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 35

gagcggcagc gtggcaagg 19

<210> SEQ ID NO 36
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 36

ggcaaatgtt ggtgacaggg 20

<210> SEQ ID NO 37
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 37

acatggacat ggccgactac 20

<210> SEQ ID NO 38
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 38

ctcgaggcac cgtagtgttt 20

<210> SEQ ID NO 39
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 39

cacatgggca ccatgtttga 20

<210> SEQ ID NO 40
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 40

aagggctggt gatggatgaa 20

<210> SEQ ID NO 41
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 41

actcctccac ctttgacgct 20

<210> SEQ ID NO 42
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 42

ggtccaccac cctgttgc 18

<210> SEQ ID NO 43
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 43

cattctcctt taacctgttg acga 24

```
<210> SEQ ID NO 44
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 44

aggacagcca tagagacaag ggca                                             24


<210> SEQ ID NO 45
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 45

agtataatgt tgagtaaatg tggtgaat                                         28


<210> SEQ ID NO 46
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 46

ccagtgatgc agctggccct ac                                               22


<210> SEQ ID NO 47
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 47

ggcaggtcta tgcataagga gcacc                                            25


<210> SEQ ID NO 48
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 48

tttttttttt tttttcaaac tgctaggatt acaggc                                36


<210> SEQ ID NO 49
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 49

tttttttttt ttttt                                                       15


<210> SEQ ID NO 50
```

<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 50

aagcctggcc tacatggt 18

<210> SEQ ID NO 51
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 51

ttttgtgaga gtgagactca gtcttaaaaa 30

<210> SEQ ID NO 52
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 52

tttttttttt tcaaccacta atcaactttc tgc 33

<210> SEQ ID NO 53
<211> LENGTH: 11
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 53

tttttttttt t 11

<210> SEQ ID NO 54
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 54

tttttttttt ttttttttcc tccctccttc tccatgta 38

<210> SEQ ID NO 55
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 55

tttttttttt tttttttt 18

<210> SEQ ID NO 56
<211> LENGTH: 21

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 56

ttttttgcc cattactcca t 21

<210> SEQ ID NO 57
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 57

gcctgcattt tatctctgtc tcgtgg 26

<210> SEQ ID NO 58
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 58

gaaggtggga aacagccaga tcaga 25

<210> SEQ ID NO 59
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 59

agagccatga cagggaataa gactaga 27

<210> SEQ ID NO 60
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 60

tgggctatgt gcatggagct t 21

<210> SEQ ID NO 61
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 61

ctgaagcaca gtgcttaccc at 22

<210> SEQ ID NO 62
<211> LENGTH: 28
<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 62

gccacaacat agtaaacgaa gaagggca 28

<210> SEQ ID NO 63
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 63

ctgtgatgcc ctacattgat ctgatttacc ta 32

<210> SEQ ID NO 64
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 64

ctgggaagtg gtgaggaggc atttt 25

<210> SEQ ID NO 65
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 65

gtatgtacca cccagcttaa cgaatgctc 29

<210> SEQ ID NO 66
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 66

cacacaggag ccacccaagg c 21

<210> SEQ ID NO 67
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 67

ccaattctgt ttctttcctt ccaggca 27

<210> SEQ ID NO 68
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 68

aaccagcctg ggtcagggtg ag 22

<210> SEQ ID NO 69
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 69

ttttttttat tccctatcta gccattagaa ccaca 35

<210> SEQ ID NO 70
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 70

tttttttttt tttttaggac agccatagag acaagggca 39

<210> SEQ ID NO 71
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 71

ccagtgatgc agctggccct ac 22

<210> SEQ ID NO 72
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 72

tttttttttt tttttttttt gttgagagag tcgatgttca ctcca 45

<210> SEQ ID NO 73
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 73

tttttttttt ttttttctc ctttcagctc tgtccgatc 39

<210> SEQ ID NO 74
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 74

tgtggtccaa acagggaaga gata 24

<210> SEQ ID NO 75
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 75

ttcatctgta ccacggcatc ataggta 27

<210> SEQ ID NO 76
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 76

ttacaatagc aatgacctgg aaccaatcc 29

<210> SEQ ID NO 77
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 77

gtgcaaccac aaacaattag gaacctgt 28

<210> SEQ ID NO 78
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 78

agcccaggag gcagcagttg c 21

<210> SEQ ID NO 79
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 79

gtcttcctaa aggagtgact gtttgcatta tcat 34

<210> SEQ ID NO 80
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

primer

<400> SEQUENCE: 80

ttgagcctgg gaggctgcg 19

<210> SEQ ID NO 81
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 81

tagcttcttc attcggtctc agtccactt 29

<210> SEQ ID NO 82
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 82

tcatttttgt agagcctgag gagtgtcca 29

<210> SEQ ID NO 83
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 83

gatttacctg ccctacaaac tttaggaggt gg 32

<210> SEQ ID NO 84
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 84

gttaccttct gtggaattaa gtggcagaac t 31

<210> SEQ ID NO 85
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 85

gacagagttt caccatgtta gccaggc 27

<210> SEQ ID NO 86
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 86

agggaccaga gccatgacag gg 22

<210> SEQ ID NO 87
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 87

tgacaagagc ttcatcccaa gaggc 25

<210> SEQ ID NO 88
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 88

tttcattggc ttcgactgtt ttcatcc 27

<210> SEQ ID NO 89
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 89

acaggatgaa gtggacgtgg aacctt 26

<210> SEQ ID NO 90
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 90

attatgtaaa gtcaggatca aagtctggct tcc 33

<210> SEQ ID NO 91
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 91

tcccttctga gaatatggct ccttgaag 28

<210> SEQ ID NO 92
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 92

aaatgaaagt ccctatcagg ccacctg 27

<210> SEQ ID NO 93
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 93

tctttctccc ccacacctcc atagaata 28

<210> SEQ ID NO 94
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 94

catccacaat tccaacaact tacgatgaag 30

<210> SEQ ID NO 95
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 95

ctatttaggc tctggctgct cttgcaa 27

<210> SEQ ID NO 96
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 96

ttacaatagc aatgacctgg aaccaatcc 29

<210> SEQ ID NO 97
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 97

aatggcaggc actggaatt 19

<210> SEQ ID NO 98
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 98

gtgcaaccac aaacaattag gaacctgt 28

<210> SEQ ID NO 99
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 99

agcccaggag gcagcagttg c 21

<210> SEQ ID NO 100
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 100

tatgaagtga aggccagaaa cga 23

<210> SEQ ID NO 101
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 101

acatgaaaaa caaagcaact ccaac 25

<210> SEQ ID NO 102
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 102

ttgagcctgg gaggctgcg 19

<210> SEQ ID NO 103
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 103

atgctggttg ctggtttatt cta 23

<210> SEQ ID NO 104
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 104

gagttgggca tgatgccttt 20

<210> SEQ ID NO 105
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 105

tcatttttgt agagcctgag gagtgtcca 29

<210> SEQ ID NO 106
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 106

caagaatgct accggcacaa 20

<210> SEQ ID NO 107
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 107

tgaatcctgg ctctgctaaa gc 22

<210> SEQ ID NO 108
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 108

accactggga gcttaagtaa aggg 24

<210> SEQ ID NO 109
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 109

tcaagttttc ccctactgag aagaat 26

<210> SEQ ID NO 110
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 110

gttaccttct gtggaattaa gtggcagaac t 31

<210> SEQ ID NO 111
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 111

ttttatccca gggattccag 20

<210> SEQ ID NO 112
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 112

gaaattcatc ccaacaagcc acacc 25

<210> SEQ ID NO 113
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 113

gacagagttt caccatgtta gccaggc 27

<210> SEQ ID NO 114
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 114

aagatgttca ggccgggc 18

<210> SEQ ID NO 115
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 115

gtagtggagg cttctcacat gtca 24

<210> SEQ ID NO 116
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 116

gcagcccagg agtcagaaac 20

<210> SEQ ID NO 117
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 117

ggatccattt atacacacca tgctt 25

<210> SEQ ID NO 118
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 118

gattcagacc atatcactgg cact 24

<210> SEQ ID NO 119
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 119

tcattgccgt cagagttact gttatta 27

<210> SEQ ID NO 120
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 120

cctgcttcaa tcctctccga 20

<210> SEQ ID NO 121
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 121

gtgaaaactc tgagcaagtg ttgtaatt 28

<210> SEQ ID NO 122
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 122

tttcattggc ttcgactgtt ttcatcc 27

<210> SEQ ID NO 123
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 123

ggatcccatg tgtcaccagg 20

<210> SEQ ID NO 124
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 124

cccgtgtcca tgtgttctca 20

<210> SEQ ID NO 125
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 125

cttcgtcaga tggatagatt gcaa 24

<210> SEQ ID NO 126
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 126

gttcttccat ttgtttgtgt cctct 25

<210> SEQ ID NO 127
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 127

tgctgacttg atcctggtgg 20

<210> SEQ ID NO 128
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 128

tcttagttat ttcttgtctc ctgctagc 28

<210> SEQ ID NO 129

<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 129

tctttgtagg tctctaagaa cttgctttat 30

<210> SEQ ID NO 130
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 130

attatgtaaa gtcaggatca aagtctggct tcc 33

<210> SEQ ID NO 131
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 131

tggtgccatg tttgaaagtt ctt 23

<210> SEQ ID NO 132
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 132

atgtgctttg attttgtgtg ttgat 25

<210> SEQ ID NO 133
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 133

ccacgtggat aatttgcatg taa 23

<210> SEQ ID NO 134
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 134

ttgaaagagt aagtagaagc gcagc 25

<210> SEQ ID NO 135
<211> LENGTH: 27

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 135

tgagcaggga gtatagagaa taaggat 27

<210> SEQ ID NO 136
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 136

tcaatctaga gacctcatac atttttagct 30

<210> SEQ ID NO 137
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 137

gagaaaacta gaattgctcc aggtaaa 27

<210> SEQ ID NO 138
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 138

aaatgaaagt ccctatcagg ccacctg 27

<210> SEQ ID NO 139
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 139

ttgcagctac ctaatacatc taacatcc 28

<210> SEQ ID NO 140
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 140

gctagtgcca ttgaagatca atttta 26

<210> SEQ ID NO 141
<211> LENGTH: 25
<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 141

aatattggtc ccttaagttc cctca 25

<210> SEQ ID NO 142
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 142

acatggagaa ggagggagga g 21

<210> SEQ ID NO 143
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 143

gctgactcag ctctccccac 20

<210> SEQ ID NO 144
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 144

accagtatga gttagtctct ggagctc 27

<210> SEQ ID NO 145
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 145

catccacaat tccaacaact tacgatgaag 30

<210> SEQ ID NO 146
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 146

atttcaatga ccagcccaca a 21

<210> SEQ ID NO 147
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

-continued

```
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 147

cattggaatc accagggagc                                          20


<210> SEQ ID NO 148
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 148

ttacttctct gctctgttat tggatactg                                29


<210> SEQ ID NO 149
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 149

ctccctggca attttcttgc                                          20


<210> SEQ ID NO 150
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 150

ttacaaagca ttattgtcat tactgcat                                 28


<210> SEQ ID NO 151
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 151

ctgtgacttt gcccattgtt taga                                     24


<210> SEQ ID NO 152
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 152

cagttcagtg tctccatcac acccagygta gggccagctg catcactggc ac      52
```

What is claimed is:

1. A method of administering a statin dose to a human, comprising:

obtaining a nucleic acid-containing test sample from a human, wherein the human is in need of statin therapy;

conducting at least one genotyping assay of the sample so as to obtain at least the genotype data at position rs35599367 of CYP3A4 in the sample, wherein the at least one genotyping assay comprises hybridizing a tagged primer to DNA in the nucleic acid-containing test sample, amplifying the hybridized DNA and detecting the tagged primer;

detecting a T allele at position rs35599367 of CYP3A4; and administering a statin dose to the human comprising a TT or TC genotype at position rs35599367 of CYP3A4 of less than 20 mg per day wherein the statin administered is a statin that depends on CYP3A4 for elimination.

2. The method of claim **1**, which further comprises a step of predicting cholesterol control outcome.

3. The method of claim **1**, which further comprises identifying at least one additional biomarker of statin metabolism.

4. The method of claim **1**, wherein said nucleic acid is a nucleic acid extract from a biological sample from said human.

5. The method of claim **4**, in which said sample is blood, saliva, or buccal cells.

6. A method of administering a statin dose to a human, comprising:

obtaining a nucleic acid-containing test sample from a human, wherein the human is in need of statin therapy;

conducting at least one genotyping assay of the sample so as to obtain at least the genotype data at position rs35599367 of CYP3A4 in the sample, wherein the at least one genotyping assay comprises hybridizing a tagged primer to DNA in the nucleic acid-containing test sample, amplifying the hybridized DNA, and detecting the tagged primer;

detecting a C allele at position rs35599367 of CYP3A4; and

administering a statin dose to the human comprising a CC genotype at position rs35599367 of CYP3A4 of more than 40 mg per day, wherein the statin administered is a statin that depends on CYP3A4 for elimination.

7. The method of claim **6**, which further comprises a step of predicting cholesterol control outcome.

8. The method of claim **6**, which further comprises identifying at least one additional biomarker of statin metabolism.

9. The method of claim **6**, wherein said nucleic acid is a nucleic acid extract from a biological sample from said human.

10. The method of claim **9**, in which said sample is blood, saliva, or buccal cells.

11. The method of claim **1**, wherein one or more statins are given, and the one or more statins is selected from the group consisting of atorvastatin, lovastatin, and simvastatin.

12. The method of claim **6**, wherein one or more statins are given, and the one or more statins is selected from the group consisting of atorvastatin, lovastatin, and simvastatin.

* * * * *